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Utilization of cellulose and gallic acid by litter inhabiting fungi and its possible implication in litter decomposition of a tropical deciduous forest

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With 5 figures

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1. Introduction

Cellulose is the most abundant carbon source in tree-leaf litter. Therefore, the successful colonization of fungi on leaf litter in part depends on their cellulolytic ability (CHESTERS, 1960, GARRETT, 1966, RAI, 1970). Since the microbial cells are impermeable to the cellulose molecule the organism must secrete extracellular enzymes to break down the complex material into simpler available forms. The extracellular catalysts act hydrolytically converting the insoluble material into soluble sugars that penetrate the cell membrane. The cellulase complex is inducible in most microorganisms and is synthesized in the presence of cellulose or carbohydrates that are structurally similar to the polysaccharide or its sugar-containing products like cellobiose and some other simple carbohydrates containing glucose in the molecule. The active organisms have catabolic repression as the regulatory mechanism of the enzyme production. There are a number of reports on the cellulolytic activity of saprophytic fungi (TRIBE, 1966, DOMSCH & GAMS, 1969, RAI, 1970, DWIVEDI & SINGH, 1974, TRIGIANO & FERGUS, 1979). However, their role in litter decomposition of a tropical dry deciduous forest has not been adequately studied.

In the present study, therefore, the dominant litter-inhabiting fungi have been tested for their ability to degrade cellulose and to produce cellulase enzyme *in vitro*. The utilization of soluble cellulose by the test fungi in culture medium and the influence of different nitrogen sources on their cellulolytic activity has also been determined.

Gallic acid (trihydroxy benzoic acid) is widely distributed in woody plants and in their litter as a component of hydrolysable tannin (JURD, 1962). Tannins decompose slowly in soil and have, with some exceptions, generally been found to be inhibitory to fungi (COOK TAUBENHAUS, 1911). Although hydrolysable tannins decompose more readily than condensed tannins (MUSGRAVE, 1948, LEWIS & STARKEY, 1968) gallic acid has been shown to be inhibitory to some basidiomycetes and microfungi of leaves and soil (LINDEBERG, 1949; DIX, 1974, 1979). There is also evidence that utilization of compounds such as glucose and lysine is inhibited by tannins which are present in litter (BASARABA, 1966, BENOIT *et al.*, 1968). Thus it is clear that the abundance of hydrolysable tannins in nature and the biological oxidation of gallic acid may affect the growth of fungal populations in litter and also the fungal decomposition of litter. In view of the above, experiments were also performed to determine the growth inhibition of the dominant litter-inhabiting fungi by gallic acid. The polyphenoloxidase activity and the ability of the selected fungi to utilize the gallic acid in the culture medium were also studied.

2. Materials and methods

2.0 Isolation of fungi

The dominant litter-inhabiting fungi selected for the following experiments were *Acremonium furcatum* (F. *et* V. MOREAU) ex W. GAMS, *Aspergillus niger* VAN TIEGHEM, *Cladosporium cladosporioides* (FRESEN.) DE VRIES, *Curvularia lunata* var. *aeria* (BATISTA, LIMA & VASCONCELOS) ELLIS, *Mortierella subtilissima* OUDEMANS, *Myrothecium advena* SACC., *Pestalotia macrotricha* KLEB., *Penicillium rubrum* STOLL, *Robillarda phragmitis* CUNNELL and *Trichoderma harzianum* RIFAI. These fungi were isolated by the method described by RAI & SRIVASTAVA (1981, 1982).

2.1. Decomposition of filter paper (C_1 cellulase activity) by the dominant litter-inhabiting fungi as influenced by different nitrogen sources

The decomposition of an insoluble form of cellulose was tested by the method described by GARRETT (1962) and FORBES & DICKINSON (1977). Wads of 10 filter papers (Whatman No. 1, mean oven dry mass approximately 5 g) were oven dried, weighed and placed in each of a number of 250 ml conical flasks with 20 ml of nutrient solution of the following composition: $NaNO_3$ 2 g, KCl 1 g, KH_2O_4 1 g, $MgSO_4 \times 7H_2O$ 0.5 g, $FeSO_4 \times 7H_2O$ 0.01 g, yeast extract 0.5 g, distilled water 1,000 ml (*p*H adjusted to 5.5). To study the effect of different nitrogen sources, $NaNO_3$ was substituted singly with $NaNO_2$, NH_4NO_3, $(NH_4)_2SO_4$ and urea in the basal medium. The concentrations of the different nitrogen compounds were adjusted so as to give an amount of nitrogen equivalent to that present in 2 g $NaNO_3$. The flasks were autoclaved for 15 min at 10.34 Newtons per cm^2 (1.034×10^5 Pascals) and were inoculated centrally on the filter paper wads with an 8 mm agar disc of mycelium of the individual test fungi grown for 5 d at 25 ± 2 °C on Czapek-Dox + 0.05 % yeast extract agar medium. Some flasks were inoculated with a sterile 8 mm agar disc to act as a control. The flasks were sealed with cellophane to prevent excessive water loss and were incubated at 25 ± 2 °C for 28 d. After the incubation, the filter paper wads were removed and dried at 80 °C (the filter paper was neither washed nor was the mycelium removed). The difference between the initial and final mass ($m_{xi} - m_{xf}$) was calculated which represents only that proportion of cellulose actually respired by the litter inhabiting fungi, and does not include the proportions converted into fungal biomass (GARRETT, 1962) was taken to be loss due to respiratory CO_2 since the amount incorporated into the mycelium was not assessed. The mass of the minerals in the wetting solution was substracted from the final mass (m_{xf}).

2.2. Utilization of carboxymethyl cellulose (CMC) by the dominant litter-inhabiting fungi as influenced by different nitrogen sources

Utilization of soluble cellulose by the test fungi was determined by growing the organisms in a medium containing $NaNO_3$ 2 g, KH_2PO_4 1 g, KCl 0.5 g, $MgSO_4 \times 7H_2O$ 0.5 g, $FeSO_4 \times 7H_2O$ 0.01 g, yeast extract 0.05 g, carboxymethyl cellulose (CMC) 10 g, distilled water 1,000 ml (*p*H adjusted at 5.5). 4 different nitrogen sources were used in the basal nutrient medium in place of $NaNO_3$ to study the effect of different nitrogen sources as described above. 50 ml of the medium was dispensed into each of 250 ml conical flasks and were autoclaved before (sect. 2.1). An 8-mm agar-mycelium disc of the individual test fungi, cut from the actively growing colonies on Czapek-Dox + 0.05 % yeast extract medium, was inoculated in the medium aseptically in triplicate. The flasks, inoculated with a sterile 8-mm agar disc served as control. All the flasks were incubated at 25 ± 2 °C for 14 d. At the end of incubation period the mycelium from the culture flasks was filtered through oven dried and weighed filter paper. The mycelium and the filter paper used to harvest the cultures were washed twice with small volumes of warm distilled water. They were then oven dried at 80 °C in a hot air oven for 4 d and weighed after cooling in a desiccator.

2.3. C_x cellulase activity of the dominant litter-inhabiting fungi as influenced by different nitrogen sources

An aliquot of the culture filtrate of the individual fungi, obtained from the above experiment (2.2), was centrifuged at 3.000 rpm for 5 min and the clear supernatant liquid was transferred to a sterile screw cap tube and stored at 2 °C for enzyme assay. The enzyme activity was determined by transferring 1 ml culture filtrate of an individual fungus to a-20 mm test tube containing 9 ml 1 % CMC-citrate-buffer solution (*p*H 5.0–5.2). After incubation at 40 °C for 1 h, 1 ml of the mixture and 3 ml dinitrosalicylic acid reagent were boiled together for 15 min. The optical densities of the solution were determined by a Systronics Spectro Colorimeter 103 at 550 nm by the following method; the reaction mixture of the uninoculated control being used to set transmittance at 100 %. The optical densities of standard solutions of D-glucose of various concentrations (0, 0.1, 0.2, 0.4 and 1 mg per ml) were determined. These were used to construct a graph of optical density as related to mg of glucose per ml. The amount of reducing sugar (RS) produced by 1 ml of the fungal filtrate acting on the CMC-citrate substratum for 1 h at 40 °C was calculated from the glucose standard graph. The C_x cellulase activity of the cell-free fungal filtrates was expressed in terms of total reducing sugars, designated as RS, and is expressed as μg RS ml^{-1}.

To test the inducible nature of enzyme production, fungi were grown in the medium containing glucose as sole source of carbon in place of CMC and the enzyme assay was done as described above. Final *p*H of the culture filtrates was determined by an Elico-*p*H meter.

2.4. Effect of gallic acid on growth of the dominant litter-inhabiting fungi

The standard inocula (5 mm disc), cut from the actively growing colonies, were transferred to the Petri dishes each containing 20 ml Czapek-Dox agar medium supplemented with 0.5 % (m/v) gallic acid. This medium was prepared from an appropriate concentration of acid solution in distilled water sterilized by Seitz filter. The sterile acid solution was then added to sterilized and cooled Czapek-Dox agar. The controls were prepared by transferring the standardized fungal inocula to the Petri dishes containing Czapek-Dox agar only. The colony diameter of the test fungi in both treated and control plates was recorded after 3 and 6 d of incubation at 25 ± 2 °C in diffused fluorescent light. Per cent inhibition of radial growth of the test fungi was calculated by comparing the increase in colony diameter of the treated plates with that of the control. The cultures were also examined for the development of brown pigmentation in the medium.

2.5. Utilization of gallic acid by the dominant litter-inhabiting fungi

The growth of the dominant litter-inhabiting fungi was studied in liquid medium containing 0.5 % (m/v) gallic acid (as a sole source of carbon) and $(NH_4)_2\ SO_4$ 2 g, KCl 0.5 g, $MgSO_4 \times 7H_2O$ 0.5 g, KH_2PO_4 0.5 g, Fe^{+++} trace, distilled water 1,000 ml (*p*H adjusted to 5.5 with NaOH). The medium was sterilized by Seitz filtration. 50 ml of sterilized medium was taken into each of 250 ml conical flasks and inoculated with one ml of standardized spore suspension ($5 \times 10^5 \times ml^{-1}$) of the test fungi, individually in replicates. The flasks were incubated at 25 ± 2 °C for 8 d after which the mycelia were filtered on oven-dried and weighed filter papers. Mycelial dry mass of the test fungi was determined after drying the samples at 80 °C for 2 d.

2.6. Determination of polyphenoloxidase activity of the dominant litter-inhabiting fungi

Polyphenoloxidase activity of the dominant litter-inhabiting fungi was determined during incubation of their mycelia in 0.5 % (m/v) gallic acid solution in distilled water (*p*H adjusted to 5.5) for 24 h at 25 ± 1°C. The fungi were grown individually in liquid Czapek-Dox medium until a large enough mycelium mat was formed which was then collected on filter paper and was thoroughly washed with sterile distilled water under aseptic conditions. The mycelium mat was then introduced into a 250 ml conical flask containing 50 ml acid solution. Reading of absorbance of the fungus inoculated acid solution was taken at 445 nm on a Systronic Spectrocolorimeter 103 against uninoculated acid blanks. After the experiment the mycelium mat was collected on oven-dried and weighed filter paper and the dry mass of the mycelium was determined. The results were expressed as absorbance change per mg dry mass of mycelium per hour. The polyphenoloxidase activity measured by absorbance could also include external physical absorbance to mycelia of the test fungi but this could be considered insignificant in comparison to the changes in absorbance as shown by different test species (fig. 3).

3. Results

3.1. Decomposition of filter paper (C_1 cellulase activity) by the dominant litter-inhabiting fungi as influenced by different nitrogen sources

After one week of incubation the filter paper wads, inoculated with the test fungi, were colonized by them. *Acremonium furcatum, Curvularia lunata* var. *aeria, Myrothecium advena, P. marotricha* and *T. harzianum* considerably degraded the filter paper resulting significant losses of dry mass (figs. 4 and 5). *Aspergillus niger* and *Robillarda phragmitis* degraded the filter paper less readily than the above mentioned species while *C. cladosporioides* and *P. rubrum* gave a very slight mass loss of the filter paper. There was significant variation in the mass loss of filter paper due to various nitrogen sources. However, $(NH_4)_2SO_4$ caused the maximum mass loss of filter paper in case of all the test fungi, except for *T. harzianum* where it was by $NaNo_3 \times NaNO_2$ was proved to be the poorest nitrogen source for decomposition of filter paper by all the test fungi, with the exception of *A. niger*.

3.2. Utilization of carboxymethyl cellulose (CMC) by the litter-inhabiting fungi as influenced by different nitrogen sources

All the test fungi grew in CMC medium as evidenced by their mycelial dry mass production (figs. 4 and 5). *Mortierella subtilissima* showed a nominal mycelial yield. Comparative mycelial dry weight production of the other 9 test fungi varied somewhat. The different nitrogen sources had an insignificant influence on the growth of individual test fungi. However, the lowest mycelial yield was observed with $NaNO_2$ (figs. 4 and 5).

3.3 C_x cellulase activity of the dominant litter-inhabiting fungi as influenced by different nitrogen sources

A. furcatum, C. lunata var. *aeria, M. advena, P. macrotricha* and *T. harzianum* showed strong C_x cellulase activity. *C. cladosporioides* showed weak C_x activity while *M. subtilissima* had no activity. Different nitrogen sources significantly affected C_x enzyme production of the test fungi. In general, $NaNO_3$ favoured the maximum C_x cellulase production, while $NaNO_2$ showed the minimum (figs. 4 and 5).

3.4. Effect of gallic acid on growth of the dominant litter-inhabiting fungi

The majority of the fungi tested showed inhibition in their radial growth on Czapek-Dox gallic acid agar medium (fig. 1). The maximum inhibition was recorded in case of *C. lunata* var. *aeria* followed by *C. cladosporioides* and *M. advena*. The growth inhibition of a species on Czapek-Dox-acid agar was accompanied by the accumulation of brown pigments in the culture medium, indicating substantial oxidation of the acid. The higher growth inhibition was related to the extensive browning of the culture medium. However, the growth of *T. harzianum* was inhibited in the beginning but no browning of the culture medium was noticed. *A. niger* and *P. rubrum* showed slight inhibition in their growth after some time, but this was not accompanied by accumulation of brown pigments in the medium (fig. 1).

3.5. Utilization of gallic acid by the dominant litter-inhabiting fungi

The determination of hyphal dry mass of the test fungi, grown in mineral salt solution with gallic acid, showed that *P. rubrum* and *A. niger* were most active in utilizing the gallic acid, followed by *T. harzianum* and *R. phragmitis*. *A. furcatum, M. subtilissima* and *P. macrotricha* grew poorly in the gallic acid medium. *C. cladosporioides, C. lunata* var. *aeria* and *M. advena* showed no growth in the medium supplemented with gallic acid (fig. 2).

3.6. Polyphenoloxidase activity of the dominant litter-inhabiting fungi

Polyphenoloxidase activity, determined in terms of change in absorbance of the fungus-inoculated acid solution (mg dry mass of fungal mycelium per hour) was found to vary among species tested (fig. 3). *C. cladosporioides* showed the strongest polyphenoloxidase activity while *R. phragmitis* and *T. harzianum* showed the poor activity. *A. furcatum, A. niger, M. subtilissima* and *P. rubrum* did not show any change of absorbance in the gallic acid solution inoculated with these fungi.

4. Discussion

Marked variations in the production of C_1 cellulase by the litter-inhabiting fungi were observed. *T. harzianum* showed the strongest cellulolytic ability with 14.6 % loss in mass of the cellulose while *C. cladosporioides* showed the weakest ability with only 5.15 % loss in mass of the cellulose. The order of cellulolytic ability of the strong cellulose decomposers was: *T. harzianum* > *C. lunata* var. *aeria* > *M. advena* > *A. furcatum* > *P. macrotricha*. Rest of the fungi tested were recognized as moderate or weak cellulose decomposers and their order of cellulolytic ability was: *A. niger* > *R. phragmitis* > *P. rubrum* > *C. cladosporioides*. *M. subtilissima* did not show cellulolytic ability. In a similar study Hogg (1966) tested 6 of the fungi found on beech leaves for their ability to utilize cellulose and he found that all the fungi tested had at least some cellulolytic activity. Rai (1970) tested the ability of fourteen saprophytic fungi to utilize cellulose *in vitro*. He found that the primary colonisers of decaying leaves of *Saccharum munja* utilized more of cellulose in comparison to secondary colonisers whereas the fungi which appeared during final stage of decomposition had the least cellulolytic ability.

There seemed to be little or no relationship between the amount of mycelium produced in the CMC-medium and the amount of reducing sugars produced in the cell-free enzyme tests (figs. 4 and 5). The small amount of mycelium produced by *M. subtilissima* in CMC-medium might be due to

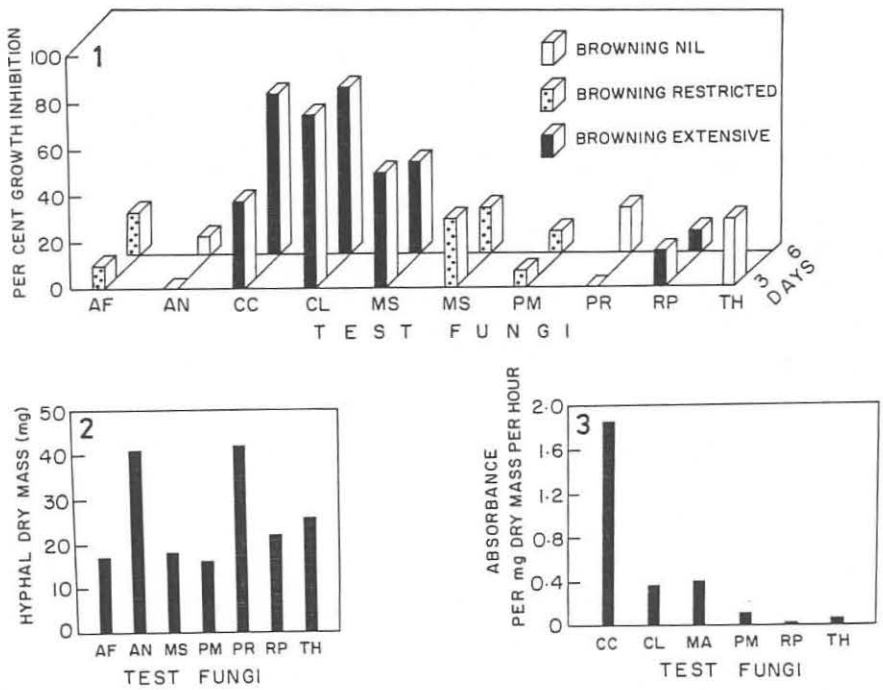

Fig. 1. Effect of gallic acid on the growth of litter-inhabiting fungi. AF *Acremonium furcatum* (F. *et* V. MORÉAU) *ex* W. GAMS, AN *Aspergillus niger* VAN TIEGHEM, CC *Cladosporium cladosporioides* (FRESEN.) DE VRIES, CL *Curvularia lunata* var. *aeria* (BATISTA, LIMA & VASEONCELOS), ELLIS, MA *Myrothecium advena* SACC., MS *Mortierella subtilissima* OUDEMANS, PM *Pestalotia macrotricha* KLEB., PR *Penicillium rubrum* STOLL, RP *Robillarda phragmitis* CUNNELL, TH *Trichoderma harzianum* RIFAI.

Fig. 2. Growth of dominant litter-inhabiting fungi in culture medium containing gallic acid (for abbreviations see fig. 1).

Fig. 3. Polyphenoloxidase activity of the dominant litter-inhabiting fungi in solution of gallic acid (for abbreviations see fig. 1).

the presence of yeast extract in the medium rather than CMC because the cell-free filtrates of this fungus could not hydrolyze CMC to reducing sugars.

Fungi which were strong C_1 cellulase producers also showed strong C_x activity. The strongest C_x cellulase producer was *A. furcatum* with 469 µg RS × ml^{-1} in the reaction mixture. The order of C_x activity amongst the strong enzyme producers was: *A. furcatum* > *M. advena* > *T. harzianum* > *C. lunata* var. *aeria* > *P. macrotricha*. The order of C_x activity of the moderate enzyme producers was: *A. niger* > *R. phragmitis* > *P. rubrum*. *A. furcatum* has already been shown to be a very good utilizer of CMC (DOMSCH & GAMS, 1969). Aspergillii are reported to be weak cellulose decomposers (NORMAN, 1931, MARSH *et al.*, 1949, BASU & GHOSE, 1960, DOMSCH, 1960), particularly in tests with crystalline forms of cellulose (WHITE *et al.*, 1948, MARSH *et al.*, 1949). Low cellulolytic activity has been shown for *C. cladosporioides* in several other studies (JENSEN, 1931, JANKE, 1949, SIU, 1951, COOKE & BAUSCH, 1957, BORUT, 1960). Several species of *Myrothecium* are known to be active decomposers of CMC (DOMSCH & GAMS, 1969) and other substrates containing cellulose (SAUNDERS *et al.*, 1948, WHITE *et al.*, 1948, KOOIMAN *et al.*, 1953, COOKE & BAUSCH, 1957, BASU & GHOSE, 1960). *Pestalotia* has also been shown to be a very good cellulose decomposer (DOMSCH & GAMS, 1969). Enzymatic studies on *Mortierella* have revealed that it is a powerful chitin and pectin decomposer (DOMSCH & GAMS, 1969, KJØLLER & STRUWE, 1980). Therefore, the dominance of *M. subtilissima* in decomposing leaf litter, despite its lack of cellulolytic activity, is possibly because a considerable amount of chitin becomes available for its growth after the death of active fungal mycelium and arthropods in the leaf litter.

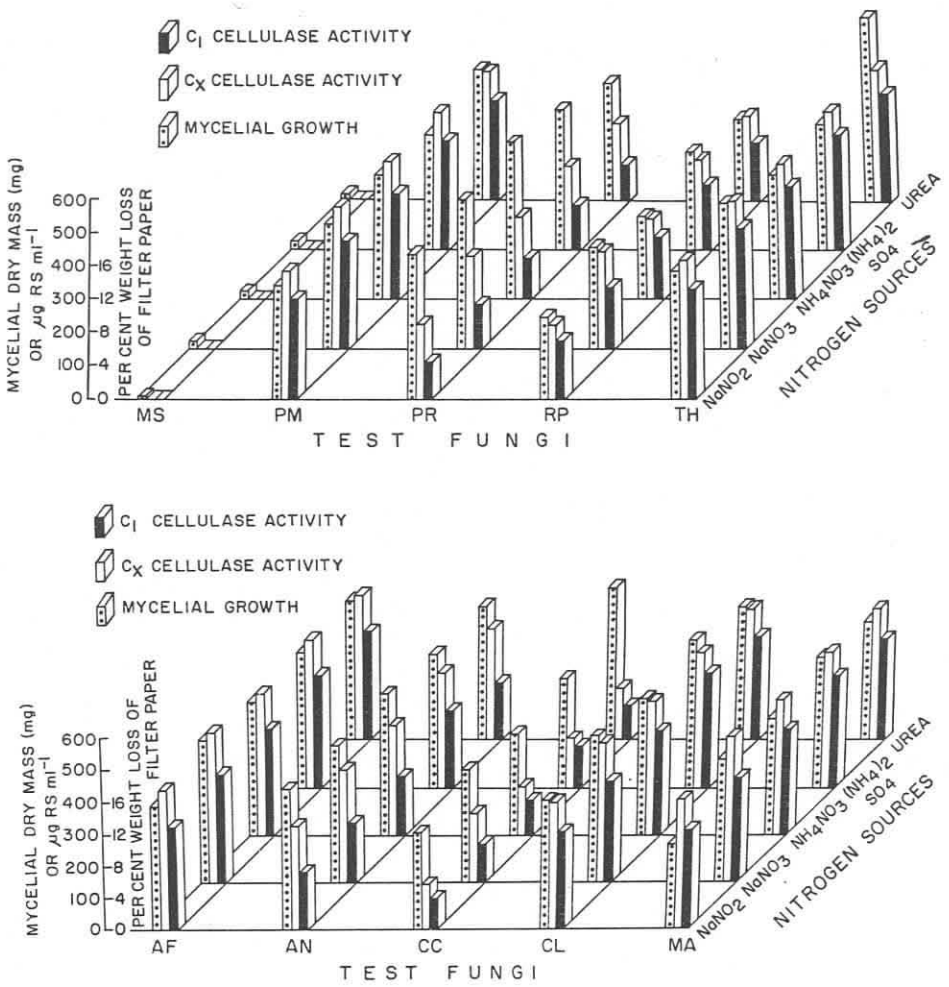

Fig. 4 & 5. *In vitro* decomposition of filter paper (C_1 cellulase activity), growth and cellulase production (C_x cellulase activity) of dominant litter-inhabiting fungi as influenced by different nitrogen sources (for abbreviations see fig. 1). RS Reducing sugars.

No C_x activity was observed in cell-free filtrates of the fungi grown in a medium containing glucose in place of CMC which indicates that the C_x cellulase is an induced enzyme produced by the selected fungi in the presence of cellulose (BREUIL & KUSHNER, 1976, ALABI & NAQVI, 1977, DENISON & KOEHN, 1977, FANELLI & CERVONE, 1977, SINGH & KUNENE, 1980). However, a little variation in the cellulolytic ability of fungi due to different nitrogen source (fig. 4) may be attributed chiefly to the *p*H change of the culture medium during the fungal growth (table 1).

Out of the 10 dominant litter-inhabiting fungi tested for their ability to produce C_1 and C_x cellulase enzymes, 5 viz., *A. furcatum, C. lunata* var. *aeria, M. advena, P. macrotricha* and *T. harzianum* were found to be strongly cellulolytic. These fungi also possess comparatively a better competitive colonization ability to exploit the substrate (RAI & SRIVASTAVA, 1983). Therefore, they may be presumed to play a major role in decomposition of cellulosic component of the leaf litter. Though *A. niger, C. cladosporioides, P. rubrum* and *R. phragmitis* had low cellulolytic ability, but they still play an important role in the decomposition of the leaf litter. In a similar study, TRIBE (1966) has also reported that in the dominance of an individual fungus on a cellulose film, cellulolytic ability appeared to be of secondary importance to its individual competitive characteris-

Table 1. *p*H of the culture medium after 14 d growth of the test fungi with different nitrogen sources

	Final *p*H of the culture medium				
	$NaNO_2$	$NaNO_3$	NH_4NO_3	$(NH_4)_2SO_4$	Urea
Acremonium furcatum	6.0	5.3	6.3	5.7	6.4
Aspergillus niger	6.1	6.2	4.9	4.9	6.7
Cladosporium cladosporioides	6.1	6.3	5.2	5.2	5.8
Curvularia lunata var. *aeria*	6.3	5.9	4.8	3.9	5.8
Myrothecium advena	5.4	6.0	5.5	4.7	6.3
Mortierella subtilissima	6.4	6.3	5.2	4.8	7.0
Pestolotia macrotricha	5.8	6.1	5.4	5.5	6.1
Penicillium rubrum	6.1	5.7	5.7	5.4	6.5
Robillarda phragmitis	6.7	5.9	6.0	5.5	6.5
Trichoderma harzianum	6.4	7.1	6.2	5.0	6.5

tics. The results obtained in the present study has provided preliminary evidence to explain the presence of these dominant fungi in decomposing leaf litter.

Inhibition of the fungal growth by gallic acid may be attributed to the accumulation of products of acidic oxidation by polyphenoloxidases (LINDEBERG, 1949). In the present experiment where higher growth inhibition was observed, polyphenoloxidase activity was also strong. Likewise where there was no growth inhibition, the polyphenoloxidase activity was nil. Thus there is a positive correlation between the inhibition of fungal growth by gallic acid and the polyphenoloxidase activity of the test fungi. The oxidation of simple polyhydroxyphenols by the catalytic action of polyphenoloxidases is a well known reaction which leads through intermediates to the formation of quinone compounds. These compounds polymerize to form characteristics and conspicuous brown pigments. LINDEBERG (1949) speculated these quinones for inhibitory action since some quinones inhibit respiration in microorganisms (WEBB, 1966). DIX (1979) isolated and described several substances from the oxidation of the gallic acid by *Ulocladium* sp. and *Setosphaeria rostrata*, most of which were inhibitory and in some cases even toxic in bioassay tests.

Since the polyphenoloxidases can be extracellular this reaction might be of some potential ecological significance for soil and litter microorganisms. The potential inhibitory effect of these substances appears to be most important in relation to the growth of common leaf litter-inhabiting fungi. HARRISON (1971) reported the inhibition of *Cladosporium herbarum* and *Epicoccum nigrum* by tannins from oak litter and there is evidence that the colonization of the leaves of woody plant species may be restricted whilst free gallic acid exists in the phylloplane (IRVINE *et al.*, 1972). COWLEY & WHITTINGHAM (1961) and HARRISON (1971) found several soil fungi inhibited by tannins. HERING (1963) demonstrated that *Cladosporium* spreads from inocula over the surface of sterilized leaves placed in the L and F layers of a forest soil. The leaf-inhabiting species are commonly isolated from tree litter and some develop their perfect stages there (HOGG & HUDSON, 1966). But the role of these species in the decomposition of litter, where growth may be restricted due to the release of gallic acid and possibly other hydroxyphenols from the breakdown of other aromatic polymers during decomposition, is still not fully undestood. Therefore, detailed study on the microbial utilization and breakdown of the aromatic polymers constituting litter is needed.

The growth inhibition of fungi in litter may, however, depend upon a number of variable factors affecting concentration of the inhibitory substances. Some free acid may exist in the litter immediately following leaf fall, though the level may be low (IRVINE *et al.*, 1978), and most will arise subsequently from the microbial breakdown of tannins. The substrate concentration will, therefore, depend upon the rate of breakdown of tannins and the rate of removal of the acid by oxidation in alternative pathways. Other important factors affecting concentration of the inhibitory substances are polyphenoloxidase activity which varies with different species (fig. 3), the possibility of removal of the inhibitory products through microbial breakdown, and a tendency to form polymers by them which may render them innocuous (DIX, 1979).

LEWIS & STARKEY (1969) found that when hydrolysable tannin was decomposed by some *Penicillium* species, gallic acid was liberated which was then decomposed without the formation of phenolics in the medium. In the present study *P. rubrum* and *A. niger* were found to be tolerant to

gallic acid which could be correlated with lack of their polyphenoloxidase activity and their growth in the presence of gallic acid. These observations suggest that these species of *Penicillium* and *Aspergillus* are important amongst the tested dominant litter-inhabiting fungi for the decomposition of plant litter under the tropical deciduous condition.

5. Acknowledgements

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Synopsis: *Original scientific paper*

RAI, B., R. S. UPADHYAY & A. K. SRIVASTAVA, 1988. Utilization of cellulose and gallic acid by litter inhabiting fungi and its possible implication in litter decomposition of a tropical deciduous forest. Pedobiologia **32**, 157–165.

The degradation of cellulose and gallic acid by some dominant litter inhabiting fungi of a tropical dry deciduous forest has been studied. The utilization of cellulose and influence of different nitrogen sources on their cellulolytic activity has also been determined. *Acremonium furcatum, Curvularia lunata* var. *aeria, Myrothecium advena, Pestalotia macrotricha* and *Trichoderma harzianum* significantly degraded the cellulose *in vitro*. Amongst the nitrogen sources ammonium sulphate was the best source accelerating the cellulose degradation. These fungi grew in carboxymethyl cellulose medium. *A. furcatum, C. lunata* var. *aeria, M. advena, P. macrotricha* and *T. harzianum* possessed strong C_1 and C_x cellulase activity.

The ability of these fungi to utilize gallic acid and to produce polyphenoloxidase was also tested *in vitro*. The majority of the fungi showed inhibition of their radial growth when the medium was supplemented with gallic acid. The maximum inhibition was recorded in case of *C. lunata* var. *aeria* followed by *Cladosporium cladosporioides* and *Myrothecium advena*. *P. rubrum* and *Aspergillus niger* were most active in utilizing the gallic acid followed by *Trichoderma harzianum* and *Robillarda phragmitis*. *C. cladosporioides, C. lunata* var. *aeria* and *M. advena* could not utilize the gallic acid. Polyphenoloxidase activity varied amongst the test fungi. Except for *A. furcatum, A. niger, M. subtilissima* and *P. rubrum* the fungi tested showed polyphenoloxidase activity. *P. rubrum* and *A. niger* were tolerant to gallic acid and grew on the culture medium in presence of the acid probably due to their lack of polyphenoloxidase.

Key words: Cellulose, deciduous forest, decomposition, fungi, gallic acid, litter, polyphenoloxidase activity.

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